Supplemental Material

Appendix 1: Additional information for methods section

Study design and population

The RED study [NTC02682563] was a randomized, double-blind, comparator-controlled intervention trial, which was designed to assess the kidney hemodynamic effects of 12-week of the SGLT2-inhibitor dapagliflozin compared to the sulfonylurea gliclazide. The current analysis concerns a prespecified analysis. The extensive trial protocol has been published previously (15). Eligible participants were men or postmenopausal women, aged between 35 and 75, diagnosed with T2D with an HbA1c of 6.5% to 9.0% and BMI >25 kg/m^2 as described earlier (15). All participants were treated with metformin monotherapy at a stable dose for at least 3 months. Blood pressure was under control (i.e., <140/90 mm Hg) and macro-albuminuria (i.e., ACR >300mg/g) was not allowed; in case of previously diagnosed hypertension and/or albuminuria, treatment included at least a stable dose of a renin-angiotensin system (RAS) inhibitor for ≥ 3 months. Exclusion criteria included instable thyroid disease, a history of rapid progressing kidney disease, malignant disease, an estimated glomerular filtration rate (GFR) <60 mL/min/1.73m^2, urinary retention, (re)current urinary tract or genital infection, diabetic ketoacidosis, cardiovascular events within 6 months prior to inclusion, use of NSAIDS or diuretics that could not be stopped 3 months prior to and during the intervention period.

Randomization, intervention, and outcome measurements

Participants were randomly assigned to dapagliflozin 10 mg daily or gliclazide 30 mg daily by using encapsulated tablets (**Supplemental Figure 1A**); participants and investigators remained blinded until database lock. The primary end points were treatment-induced changes in measured GFR and effective renal plasma flow (ERPF) from baseline to week 12 of dapagliflozin versus gliclazide, measured in fasting conditions and during clamped euglycemia and hyperglycemia. Measurement of uric acidwas a pre-specified exploratory endpoint.

Study protocol

Participants were admitted to the Clinical Research Unit of the Amsterdam University Medical Centers, location VUMC in Amsterdam, the Netherlands. Medical history and medication were recorded. Body weight and height were measured, and BMI calculated. Lean body mass was assessed by single-frequency BIA bioelectrical impedance analyzer (Maltron BF-906; Maltron International, Essex, U.K.). Systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate were measured three times consecutively by an automated oscillometric device (Dinamap; GE Healthcare, Little Chalfont, U.K) over the brachial artery of the non-dominant arm, using the mean of the last two measurements. Blood samples were obtained for fasting outcome variables, including uric acid, sodium, glucose, creatinine, HbA1c, insulin and albumin. Hereafter, a 24hr urine to measure uric acid, sodium, glucose, albumin and creatinine, collected until 20.00 the day prior to the test day, was received and processed, after which a protocol to measure GFR and ERPF started that has been described in detail earlier (15). In short, after a bolus infusion of 22.5 mg/kg inulin (Inutest®, Fresenius Kabi Austria GmbH, Graz, Austria) and 3 mg/kg PAH (4-Aminohippuric Acid Solution 20%, Bachem Distribution Services GmbH, Weil am Rhein, Germany), continuous infusion started at respectively 675 and 320 mg/h until the testing day was over (**Supplementary material Appendix 1B**). During the study testing period, inulin was retracted from the market due to anaphylactic reactions elsewhere after which the measurement of GFR continued with iohexol (bolus 36 mg/kg, continuous infusion 906 mg/h; Omnipaque^{TM}, GE Healthcare B.V. Eindhoven, the Netherlands) (15). Following a 100 min equilibration period, blood was drawn 15 min apart to measure plasma concentrations of inulin/iohexol and PAH in steady-state fasting conditions. From 115 to 295 min, a hyperinsulinemic-euglycemic clamp was performed, with insulin (Novorapid, Novo Nordisk, Denmark) infusion at 40 mU/min·m^2 while maintaining plasma glucose at 90.1 mg/mL by variable glucose 20% infusion. During the last 90 min of the clamp, urine and blood was collected every 45 min to measure urinary and plasma inulin/iohexol, PAH, uric acid , sodium, glucose, and plasma concentrations of insulin during hyperinsulinemic-euglycemic conditions. During the last 30 min insulin sensitivity was

measured by glucose infusion rate, and corrected for lean body mass (M-value) (mg/kg_{lean}/min). The end of the clamp was followed by a 60-minute rest period to clear exogenous insulin. Then, a hyperglycemic clamp was performed from 355 to 505 min. Bolus infusion of 20% glucose solution (depending on body weight and fasting plasma glucose concentration) was followed by adjusting the rate of the 20% glucose infusion to maintain plasma glucose at at 270mg/mL After 60 min of equilibration, urine and blood was again collected every 45 min to measure urinary and plasma inulin/iohexol, PAH, uric acid, sodium, glucose, and plasma concentrations of insulin during hyperglycemic conditions. The clamp procedure was conducted at baseline and after 12 weeks of treatment with dapagliflozin to measure GFR and ERPF.

Sample size calculation

Sample size of the RED study was based on the expected between-group difference in mGFR using Stata version 11 (Breda, The Netherlands). Assuming an SD of 15.0 ml/min and considering an $\alpha = 0.05$ as significant, 19 participants per treatment arm were needed to achieve a power of $(1 - \beta)$ 80% to detect a between-group mGFR difference of 14 ml/min (89 ml/min in the gliclazide group vs. 75 ml/min in the dapagliflozin group and thus 16% difference). To achieve a power $(1 - \beta)$ of 80% to detect a dapagliflozin-induced mGFR reduction (i.e., within group) from 89 to 75 ml/min (16% difference) with an SD of 15.0 ml/min, assuming $\alpha = 0.05$ (2-sided testing) is significant, 10 participants were needed. We increased the number of subjects per group to 22 to allow a maximum dropout percentage of 15%.

UREX study

Study design and population

The UREX study (NCT03433248) was an open-label randomized cross-over intervention study that investigated 1-week mono- and combination therapy with URAT1-inhibitor benzbromarone and SGLT2-inhibitor empagliflozin on plasma uric acid and fractional excretion of uric acid. Eligible participants met the same criteria as the RED study, with two exceptions; a

combination of metformin and low-dose sulfonylurea derivative as glucose-lowering therapy was allowed and a history of gout was added to the exclusion criteria. No participants included in the studies used other uric acid-lowering agents.

Randomization, intervention, and outcome measurements

The study started with a 4-week run-in period, followed by a baseline visit. Hereafter, participants were treated for 1-week with SGLT2-inhibitor empagliflozin 25 mg once-daily, benzbromarone 100 mg once-daily or empagliflozin 25 mg once-daily plus benzbromarone 100 mg once-daily in a random order (**Supplemental Figure 1C**). Treatment periods were separated by a 4-week washout period. Primary outcome measurements were plasma uric acid and fractional excretion of uric acid.

Study protocol

Participants were admitted to the hospital to obtain medical history, medication and measurement of body weight, height, and blood pressure (Dinamap; GE Healthcare, Little Chalfont, U.K). Blood samples were obtained for fasting outcome variables, including uric acid, sodium, glucose, creatinine, HbA1c, insulin and albumin. Hereafter, a 24hr urine to measure uric acid, sodium, glucose, and creatinine, collected until 20.00 the day prior to the test day, was received and processed. Blood determinations were performed using conventional assay methods by the Department of Clinical Chemistry in the Amsterdam University Medical Centers – location VUmc as described (15). Uric acid was measured as urate with an enzymatic colorimetric test (Cobas-C501; Roche Diagnostics, Indianapolis, IN). Fractional excretions of uric acid in RED an UREX were calculated according to standardized formula.

Biochemical measurements

Blood determinations were performed using conventional assay methods by the Department of Clinical Chemistry in the Amsterdam University Medical Centers – location VUmc as described (15). Inulin and PAH concentrations were quantified using colorimetric assay after

preparation with *p*-dimethylamino-benzaldehyde for inulin and trichloroacetic acid and indole-3-acetic acid for PAH. Iohexol was measured using liquid chromatography tandem mass spectrometry (TSQ Quantiva with UHPLC Vanquish, Thermo Fisher Scientific, Waltham, MA) and was carried out at the University Medical Center Groningen. Uric acid was measured as urate with an enzymatic colorimetric test (Cobas-C501; Roche Diagnostics, Indianapolis, IN) and urine-pH was buffered to >8.0 with NaOH.

Calculations

Fractional excretions in the RED study were calculated as (Ux/P_x) x $(P_{inu/ioh}/U_{inu/ioh})$, where U_x represents the urine concentration of substance x, $P_{inu/ioh}$ the plasma concentration of inulin/iohexol and $U_{inu/ioh}$ the urine concentration of inulin/iohexol. Clearances of inulin/iohexol and PAH were based on infusion rates and timed plasma sampling, and calculated as (IR_x / P_x), where IR_x represents the infusion rate and P_x the plasma concentration of substance x. For the UREX study fractional excretions were calculated as (U_{ua}/P_{ua}) x (P_{cr}/U_{cr}), where U_{ua} represents the urinary concentration of uric acid, P_{ua} the serum concentration of uric acid, P_{cr} the serum concentration of creatinine, U_{cr} the serum concentration of creatinine.

Statistical analyses and post-hoc outcome measures

Outcome measures of this prespecified post-hoc analyses consist of plasma uric acid levels and fractional excretion of uric acid levels. Data on demographics are presented as mean ± SD if normally distributed, and median (IQR) for positively skewed variables. Continuous variables were tested for distribution and log-transformed in case of a positively skewed variables.

For the RED study, different states were compared using a repeated measure ANOVA, or in case of non-normal distribution, by using a Friedman test. Variables that correlated with plasma uric acidwere included in a multivariable linear regression to adjust for potential confounders including age, sex, BMI and kidney hemodynamics. Within group comparisons of treatment effects were analysed using paired *t*-tests or Wilcoxon signed rank test. Sample

size of the RED study was based on the expected between-group difference in mGFR using Stata version 11 (Breda, The Netherlands) as previously described (15). In the RED study there were missing data on the GFR and ERPF measurements of one patient in the dapagliflozin group. All analyses were performed without these missing data.

For the UREX study, to assess differences between groups paired *t*-test or Wilcoxon signed rank test were applied. Sample size for the UREX study was calculated as following: we expected an SGLT2-inhibitor induced reduction in fasting plasma uric acid from 320 μmol/L to 270 μmol/L with SD of 50 μmol/L; with alpha two-sided at 0.05 and power of 0.80, 10 participants needed to be included given within-individuals comparisons. All analyses were performed using SPSS version 26.0 and statistical significance was defined at a two-tailed *p*-value > 0.05. Figures were created using GraphPad Prism version 9.1.0.

Supplemental Figure 1: Study design and protocol RED and UREX

Study design and test protocol | (a) Study design RED with time in weeks. (b) Schematic overview of the test protocol with time in minutes. [a]Including 24-hr urine collections [b]Bolus infusion in the first 10 minutes. PAH, para-aminohippuric acid. (c), Study design UREX with time in weeks. R, Randomization; S, Screening visit and eligibility assessment; BL, Baseline tests performed to assess study objectives at baseline; LT, Long-term follow-up tests performed to assess study objective at end-point (i.e. after 12 weeks of intervention) ; CV, Cardiovascular measurements; M-value, Measure of insulin sensitivity; QD; once daily